

EFFECT OF DIETARY MANNAN OLIGOSACCHARIDES SUPPLEMENTATION LEVEL ON THE CARCASS CHARACTERISTICS, MEAT QUALITY AND INTESTINAL MICROBIAL ECOLOGY OF JAPANESE QUAIL (*COTURNIX JAPONICA*)

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ABSTRACT

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The effect of three different levels of mannan oligosaccharides (MOS) as a dietary supplement on carcass characteristics, meat quality and intestinal microbial ecology of growing Japanese quail (*Coturnix japonica*) was the main objective of the present experiment. A total of one hundred 1-day-old Japanese quails were randomly divided into 4 experimental groups (25 birds/ each treatment) with 3 replicates (8 birds in two replicates and 9 birds in one replicate) in each group. The birds of experimental groups were fed on four dietary treatments: 1) a basal diet without supplementation (control); 2) a basal diet with 1 g MOS/kg diet (low MOS); 3) a diet with 3 g MOS/kg diet (medium MOS); and 4) a diet with 5 g MOS/kg diet (high MOS). The experimental period extended for 42 days. The data revealed that, birds fed diets containing medium MOS level (3 g /kg feed) recorded significant ($P < 0.05$) improvements in body weight and weight gain compared with other treatment groups. Medium level of MOS supplementation increased the dressing and edible giblets percentages, while the offal's and carcass abdominal fat percentages were significantly decreased. Crude protein and moisture values of quail's meat were higher in medium MOS supplemented birds diet than in other groups, while fat and ash values were lower. Total aerobes and *E. coli* counts were not significantly differed ($P > 0.05$) between the treated groups. A significant increase in lactobacilli counts were detected in duodenum and jejunum of MOS supplemented groups diet. Birds fed medium MOS supplemented diet showed the highest lactobacillus counts. From the results of the current study, it could be concluded that medium level (3g MOS/Kg feed) of mannan oligosaccharide improve the carcass characteristics, meat quality and intestinal microbial ecology of growing Japanese quails by increasing the growth of beneficial microbes and reduction of potential pathogens.

Key words: *Mannan oligosaccharides, carcass characteristics, meat quality, intestinal microbial ecology, Japanese quails.*

INTRODUCTION

Nowadays, the efficiency of poultry to convert the feed into meat plays a key role in economics of broiler industry. Therefore, it is highly essential to improve feed efficiency of poultry to produce meat economically and also food safety is more seriously considered than before. A huge amount of antibiotics have been used to control diseases and improve performances in livestock. The use of antibiotics to promote growth and control diseases in farm animals has been the usual practice for many decades among farmers (Plail, 2006; Zeweil *et al.*, 2006; Akinleye *et al.*, 2008). But by long-term use, side effects of antibiotics occur, like residues in meat, development

of drug-resistance bacteria and reduction in the ability to cure these bacterial diseases in humans (Donoghue Dan, 2003). Many countries are either regulating the use of antibiotics in feed or setting up programs to reduce the overall use of antibiotic. Therefore, the use of probiotic and prebiotic in poultry diet has become popular as an alternative to antibiotic for animal production and health worldwide in recent years (Sahin *et al.*, 2008; Vali, 2009; Erdogan *et al.*, 2010; Skvortsova, 2010; Sahin *et al.*, 2011). One such additive that is being tested as growth promoter is the mannan oligosaccharides (MOS) of the cell wall of the yeast *Saccharomyces cerevisiae*. When MOS are incorporated in the animal feed, they can adhere to pathogenic bacteria that have type-I fimbriae and so

limit their ability to adhere to the mucosa of the digestive tract and to multiply. In addition, MOS can benefit the intestinal function by improving the height, uniformity, and integrity of the intestinal villi (Hooge, 2004; Ghosh *et al.*, 2007; Kogan and Kocher, 2007; Rehman *et al.*, 2009). Moreover, they can exert a positive effect on the immune response of the animal and the production of IgA antibodies. As a result, the replication of many pathogens is being limited and the health of the gut improves (Ghosh *et al.*, 2007, Rehman *et al.*, 2009).

The effects of prebiotic on gut microflora interact with digestive physiology and growth which can be further influenced or even determined by many other factors such as the compatibility between the diet and the prebiotic, hygiene standards and animal husbandry practices. There possibly remain many questions to be answered or barriers to be overcome so that the alternatives to antibiotics can be applied (more) successfully in the industry in future (Yang *et al.*, 2009). To maintain the intestinal microflora balance in animals it is important to prevent diseases by controlling the overgrowth of potentially pathogenic bacteria. The control of such potentially pathogens through a non antibiotic approach is urgently requested. Strategic use of these alternative compounds will help optimize growth, provided they are used in a manner that complements their modes of action (Cakir *et al.*, 2008). Therefore, the objective of the present study was to investigate the effect of different dietary levels of mannan oligosaccharides (MOS) on carcass characteristics and on intestinal microbial ecology of the growing Japanese quail (*Coturnix japonica*).

MATERIALS and METHODS

Bird and housing

This study was carried out at the quail production unit, Faculty of Veterinary Medicine, South Valley University, Egypt, during the period from May to June 2011. Chemical analyses were performed in the laboratories of the Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt. A total number of 100 one day old Japanese quail chicks were divided randomly into equal four treatments (25 birds each); each group was subdivided into three replicates (two of 8 birds and one of 9 birds /battery cage). Chicks were individually weighed to the nearest gram at the start of experiment (the mean of the initial body weight was about 9.66 ± 0.26 g), wing-banded and randomly allotted to the dietary treatments. Chicks were raised in electrically heated batteries with raised wire mesh floors and had a free access to the mash food and fresh water from nipple drinkers throughout the experiment. Light was provided for 23 h/d. Room temperature on day 0 was 35°C and decreased

approximately 2.5°C per week until 25 °C was reached, according to standard poultry rearing practices. Batteries were placed into a room provided with continuous fans for ventilation. Heating and forced ventilation system allowed room temperature to be maintained between 25 and 35 °C.

Dietary treatments

The dietary treatments were: 1) a control diet without Y-MOS supplementation; 2) a diet with a prebiotic Y-MOS at a level of 1 g/kg feed; 3) a diet with Y-MOS at a level of 3 g/kg feed, and 4) a diet with a Y-MOS at a level of 5 g/kg feed. Diets were fed in mash form. A commercial prebiotic source Y-Mos® (Nutrex, Belgium) was used in this experiment; chemical composition of the Y-MOS is presented in Table 1. Basal diet was formulated to contain the metabolized energy (ME) density (2900 kcal/kg) and crude protein (24 %) concentrations recommended by NRC (1994). Ingredients and calculated chemical compositions of the basal experimental diet are presented in Table 2. No coccidiostats or antibiotics were used during the study. Feed and water were provided *ad libitum*. All birds were kept under hygienic conditions and were subjected to a prophylactic vaccination against viral diseases.

Carcass traits

Five birds randomly selected from each group were slaughtered at the end of the experiment. The birds were fasted for 10-12 h prior slaughtering to determination of the final body weight. Carcass weight (the weight of the slaughtered birds after removal of feathers, head, and feet but including the edible giblet "liver without gall bladder, heart, skinned empty gizzard and abdominal fat") and the absolute organ weights were recorded. Relative organs weights, dressing %, offal's %, and giblet % are calculated as relative weight to live body weight.

Meat chemical composition

Different parts from the carcass as breast and thigh were sealed in polyethylene bags and frozen at -20 °C for further analysis. The meat was removed from the bones, it was homogenized and it was analyzed for crude protein, crude fat, moisture and ash, according to the guidelines of AOAC (2005).

Microbial counts

Intestinal content from the duodenum, jejunum, ileum and caecum was taken separately and immediately after slaughter in previously weighed screw-capped sterile plastic cups. Digesta was evacuated and mixed. The sealed containers were kept on ice until they were transported to the laboratory for enumeration of microbial population. The fresh mass was mixed with appropriate volume of sterile 0.1% peptone solution to prepare 1:10 dilution. Ten fold serial dilutions up to 10^7 of each sample were then prepared in 9 ml of 0.1% sterile peptone solution. Viable counts of total aerobes, *E. coli*, and lactobacilli were performed using the spread-plate technique. Total aerobes were

enumerated on nutrient agar (Oxoid) incubated aerobically at 37°C. The Eosin methylene blue (EMB) agar (Oxoid) was used for *E.coli*, incubated aerobically at 37°C. Plates were counted between 24 and 48 h after incubation. For lactobacilli, deMan, Rogosa and Sharpe (MRS) agar (Biolife) was used, and the plates were incubated in 5% CO₂ for 48h. The media plates were inoculated with 0.1ml of the sample dilutions. Three dilutions were plated for each count as appropriate (10¹, 10³ and 10⁵ for *E.coli* and 10³, 10⁵ and 10⁷ for total aerobes and lactobacilli). After incubation, colonies were counted according to colony morphology for *E.coli* (isolated colonies with dark purple centers and greenish metallic sheen) and lactobacilli (small opaque and white, compact or feathery colonies). For total aerobes all colonies were counted. Counts from two plates were averaged. Numbers of colony-forming units are expressed as log colony-forming units per gram of Digesta content.

Statistical analysis

The data were subjected to statistical analysis with one way ANOVA using SPSS program for Windows Version 13 (SPSS, 2001) to determine if variables differed between groups. Statistical significant effects were further analyzed, and means were compared using Duncan’s multiple range test (Duncan, 1955). Statistical significance was determined at *P* < 0.05.

RESULTS

Carcass traits, absolute organ weights:

Supplementation of MOS significantly altered (*p*<0.05) carcass characteristics of growing Japanese quails. The data in Table 3 indicated that, carcass weight, and dressing % of medium MOS supplemented quails diet were significantly (*p*<0.05) higher than other treatment groups. Moreover, carcass of birds fed medium MOS supplemented diet had

lower offal's weight and lower abdominal fat percentage than other groups. In addition, the relative liver and gizzard weights tended to be higher in medium MOS supplemented birds diet.

Meat chemical composition

Table 4 revealed that, the meat chemical composition of quails fed diet supplemented with medium MOS had numerically the highest average moisture (74.00±0.58) and crude protein (21.96±0.08) percentages compared to the other two treatments and the control group. Quails fed medium MOS supplemented diet displayed a significantly (*p*<0.05) lower average fat % (1.97±0.09) in their meat compared to control one. Average ash %, as well, was lower (1.3±0.06) in meat of medium MOS supplemented quails compared to other tested groups.

Microbial counts

The data presented in Table 5, display the effect of dietary MOS supplementation on the intestinal total aerobes, *E.coli* and lactobacilli counts in the different parts of the small intestine (Duodenum, Jejunum and Ileum) and the Caecum. No significant differences (*P*>0.05) were found between tested groups concerning total aerobes counts. Roughly, the count was relatively higher in quails fed MOS supplemented diets than in the control group. On the other hand, *E.coli* counts were relatively higher in control group than in MOS supplemented ones, with no significant differences (*P*>0.05). Lower lactobacilli counts were recorded for the control group than for MOS supplemented ones with a significant differences (*P*<0.05) for duodenum and jejunum counts. In-between MOS supplemented groups a significant difference (*P*<0.05) was detected between medium MOS supplemented and the other two treatments in case of duodenum and jejunum. Birds fed medium MOS supplemented diet showed the higher lactobacillus counts.

Table 1: Chemical composition (%) of mannan oligosaccharide product (Y-MOS)

Item	Y-MOS
Dry matter (DM)	95
Protein /DM%	25
Ash	6
Polysaccharides	
B-Glucanes	28
Mannan oligosaccharides (MOS)	28

Table 2: Ingredients and chemical composition of basal diet fed for Japanese quail (% , as fed-basis)

Ingredients	%
Yellow corn	55.95
Soybean meal (48 % Crude protein)	39.6
Sunflower oil	1.00
Dicalcium phosphate	1
Limestone, ground	1.5
Iodized salt	0.4
Premix*	0.25
L-lysine	0.1
DL-methionine	0.2
Calculated Chemical Composition	
Crude protein	24.04
Ether extract	2.36
Crude fiber	3.22
Calcium	0.91
Available phosphorus	0.31
Lysine	1.41
Methionene + Cystine	0.75
Metabolizable energy kcal /Kg	2937.16

* Mineral and vitamin premix, Heromix broilers (Heropharma Co., Egypt)
 Each 2.5 kg contain: 12,000000 IU Vit. A, 2,000000 Vit D3, 10 g vit. E, 2g Vit K3, 1g Vit. B1, 5g vit B2, 1.5 g Vit. B6, 10 mg Vit B12, 30 g nicotinic acid, 10 g pantothenic acid, 1g folic acid, 50 g biotin, 250 g choline chloride 50 %, 30g iron, 10 g copper, 50g zinc, 60 g manganese, 1g iodine, 0.1 g selenium, 0.1 g cobalt and carrier Caco3 to 2.5 kg

Table 3: Effects of mannan oligosaccharide (MOS) on carcass characteristics and relative organ weight (g) of growing Japanese quail

Item	Control	Low MOS	Medium MOS	High MOS	P
Live weight	193.00±2.41 ^c	204.2±4.43 ^b	214.00±2.6 ^a	188.8±5.97 ^d	0.000
Carcass weight	131.6±2.62 ^d	145.2±4.86 ^b	159.6±3.03 ^a	132.2±2.22 ^c	0.000
Dressing, %	68.18±0.84 ^c	71.1±1.88 ^{ab}	74.56±0.59 ^a	70.57±1.04 ^b	0.02
Offals weight	61.4±1.7	59.00±4.2	54.4±0.87	55.6±2.37	0.25
Offals, %	31.82±0.9 ^a	28.88±1.89 ^{ab}	25.99±0.59 ^b	29.93±1.04 ^a	0.01
Edible giblet wt	14.18±0.6	13.6±0.82	14.6±0.23	13.00±0.75	0.36
Edible giblet, %	7.34±0.26	6.68±0.24	6.83±0.18	6.88±0.34	0.45
Liver	2.97±0.11	2.57±0.16	3.16±0.06	2.97±0.19	0.06
Gizzard	1.79±0.09	1.62±0.14	1.86±0.14	1.38±0.12	0.06
Heart	1.21±0.04	1.16±0.1	1.1±0.05	1.2±0.05	0.61
Spleen	0.06±0.01	0.06±0.01	0.07±0.02	0.05±0.005	0.6
Head	6.46±0.24	6.17±0.87	5.7±0.24	5.84±0.44	0.73
Abdominal fat	1.36±0.09 ^a	1.32±0.14 ^a	0.7±0.07 ^b	1.32±0.19 ^a	0.007
Legs	2.91±0.23 ^a	2.14±0.13 ^b	2.13±0.02 ^b	2.06±0.17 ^b	0.005

Figures in the same raw with different superscript differ significantly (p< 0.05).

Values are reported as means ± SE.

n=5

n=number of birds

Offals weight= weight of (blood +feather +head+legs)

Edible Giblet weight = weight of (liver+ skinned gizzard+heart+abdominal fat)

Dressing %, offals %, giblet % , organs relative weights are calculated in relation to live weight

Table 4: Effect of MOS supplementation on carcass meat composition of Japanese quail (mean±SE)

Items	Control	Low MOS	Medium MOS	High MOS	P
Moisture	72.0±0.57	71.67±1.4	74.00±0.58	72.35±1.2	0.37
Crude protein	20.83±0.44	21.63±0.6	21.96±0.08	21.66±0.33	0.11
Fat	3.67±0.35 ^a	2.60±0.31 ^b	1.97±0.09 ^b	2.17±0.27 ^b	0.01
Ash	1.40±0.05 ^b	1.67±0.09 ^a	1.3±0.06 ^b	1.5±0.05 ^a	0.01

Figures in the same raw with different superscript differ significantly (p< 0.05).

Values are reported as means ± SE.

n=3 n=number of birds

Table 5: Effect of MOS on intestinal microbial ecology of Japanese quail (mean±SE)

Item	Control	Low MOS	Medium MOS	High MOS	P
Total aerobes					
Duodenum	10.51±0.35	9.07±1.24	11.00±0.71	9.17±0.48	0.27
Jejunum	7.94±0.38	9.97±1.34	9.32±0.80	9.89±0.34	0.34
Ileum	8.55±0.86	10.36±1.20	11.97±0.53	10.63±0.20	0.09
Caecum	9.25±0.39	9.11±1.41	9.85±1.15	10.53±0.24	0.71
Lactobacilli					
Duodenum	4.15±0.66 ^c	5.67±.38 ^b	8.56±0.27 ^a	6.96±0.36 ^b	0.001
Jejunum	4.50±0.57 ^c	6.38±0.34 ^b	8.55±0.13 ^a	6.15±0.70 ^b	0.003
Ileum	4.18±1.14	5.64±0.98	7.94±1.08	6.99±0.63	0.11
Caecum	4.93±0.77	6.28±0.48	6.59±1.02	6.25±0.46	0.42
E. Coli					
Duodenum	4.63±0.82	3.41±1.51	3.38±0.78	3.36±1.26	0.82
Jejunum	3.38±0.44	4.92±1.61	3.41±0.82	2.50±0.51	0.41
Ileum	4.97±0.54	2.66±0.71	3.27±1.28	3.53±1.30	0.47
Caecum	2.59±0.60	1.89±0.10	2.49±0.50	2.96±0.48	0.48

Figures in the same raw with different superscript differ significantly (p< 0.05).

Values are reported as means ± SE.

n=3 n=number of birds

DISCUSSION

Gibson and Roberfroid (1995) defined a prebiotic as a non-digestible food ingredient which beneficially affects the host by selectively stimulating the growth of and/or activating the metabolism of one or a limited number of health-promoting bacteria in the intestinal tract. It was hypothesized that a decrease in intestinal pathogen challenge provided by MOS would result in improvement of nutrient utilization and allocation leading to benefit in lean muscle gain and dressing percentage (Ferket, 2004). The improvements of carcass weights, dressing percentages, offal's weights and the decrease in

abdominal fat in medium MOS supplemented quails were in harmony with the results of previous studies on Japanese quails (Guclu 2003; Parlat *et al.*, 2003; Oguz and Parlat 2004; Falaki *et al.*, 2011) and broilers (Bozkurt *et al.*, 2008; Zhou *et al.*, 2009). This result could be due to decreased proliferation of pathogenic bacteria. Thus, the digestive tract remains healthy, functions more efficiently and more nutrients are available for absorption (Spring *et al.*, 2000). The decrease in abdominal fat and muscle crude fat percentages were consistent with results of Ammerman *et al.* (1989) who concluded that the addition of 0.3% oligofructose to the bird's ration decreased the percent of abdominal fat. On this

respect, Maisonnier *et al.* (2003) reported that as the incorporation of oligosaccharide in the diet, diluted the bile salt and reduced the lipid digestibility, so admission of MOS in the diet of Japanese quail may result in reduction of crude fat percentage in muscle. Ghosh *et al.* (2008) recorded significantly lower crude fat percentage in the meat of quails fed MOS at rate of 0.1% but no difference in moisture or crude protein percentages of the meat, which are in agreement with the current findings. On the other hand, Bonos *et al.* (2010) mentioned that the addition of MOS at a rate of 0.1% had no significant ($P>0.050$) effect on the meat composition in quails. Furthermore, Bozkurt *et al.* (2005); Bozkurt *et al.* (2008), Ghosh *et al.* (2008); Konca *et al.* (2009) and Sarica *et al.* (2009) reported no significant improvement in carcass yield parameters (carcass weight and carcass dressing, breast, back, legs, neck, wings and heart percentages) by MOS supplementation.

The significant increase in lactobacilli colony count in duodenum and jejunum agree with the previous studies (Oyarzabal and Conner, 1996; Xu *et al.*, 2003; Chung and Day, 2004; Mountzouris *et al.*, 2007). These studies proved that, probiotics and prebiotics could balance the intestinal microecosystem by controlling pathogenic bacteria via a competitive reaction which improves the count of beneficial bacteria. By binding with pathogenic bacteria possessing type I fimbriae (e.g. *Salmonella enteritidis*, *S. typhimurium* and *E. coli*), MOS can prevent them from attaching to the gut lining moving them through the intestine without colonization (Dawson and Pirvulescu, 1999; Spring *et al.*, 2000; Shane, 2001; Loddi *et al.*, 2002). On this respect, Baurhoo *et al.* (2007) and (2009) reported an increase of lactobacilli and bifidobacteria in the ceca of broilers due to dietary MOS, while Spring *et al.* (2000) noted a decrease of *Salmonella* in the ceca of broilers, but no difference in lactobacilli, coliforms, enterococci, and anaerobic bacteria. Moreover, Sims *et al.* (2004) found no significant difference in lactobacilli, coliforms, and *E.coli* in turkeys fed MOS, whereas Song and Li (2001) and Ghosh *et al.* (2007) reported a decrease in *E.coli* in the small intestine of broilers fed MOS. From the trend of the analysis it was indicated that MOS supplementation had an influence in reducing *E.coli* in the gut of experimental birds which was in accordance with the findings of Lon (1995), Fairchild *et al.* (2001), and Ghosh *et al.* (2007). Several mechanisms have been proposed to explain this modification in the microflora balance: competition for receptor sites, production of antimicrobial products (e.g., bacteriocins), production of volatile fatty acids, or stimulation of the host immune system (Strompfova *et al.*, 2007; Brzoska *et al.*, 2007). Bonos *et al.* (2011) found no significance difference ($P>0.05$) in birds fed different levels of MOS (0.1 and 0.2%) concerning total aerobic bacteria, coliforms or lactic acid bacteria counts in the

caecum, which are in partial agreement with our findings.

In conclusion, the current study declared that medium dietary level (3g MOS/Kg feed) of mannan oligosaccharide (MOS) improve the carcass characteristics, meat quality and intestinal microbial ecology of growing Japanese quails by increasing the growth of beneficial microbes and reduction of potential pathogens.

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تأثير مستوى سكر المنان الأحادي مضافاً في العليقة علي مواصفات الذبيحة ، جودة اللحم والبيئة الميكروبية المعوية في السمان الياباني النامي

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أجريت هذه التجربة لتقييم أثر إضافة ثلاث مستويات غذائية مختلفة من سكر المنان الأحادي (MOS) في العليقة علي مواصفات الذبيحة، جودة اللحم، والتغيرات في البيئة الميكروبية لأمعاء السمان الياباني النامي. استخدم عدد 100 طائر من السمان الياباني النامي عمر يوم تم تقسيمهما عشوائياً إلي عدد 4 مجموعات بكل منها عدد 25 طائر وقسمت كل مجموعة إلي 3 مكررات (اثنان بهما 8 طيور وواحدة بها 9 طيور). غذيت المجموعة الأولى علي العليقة الضابطة (بدون أي إضافات)، في حين غذيت المجموعة الثانية والثالثة والرابعة علي العليقة الضابطة بعد إضافة 1 جم MOS/كجم عليقه (مستوي منخفض)، و 3 جم MOS /كجم عليقه (مستوي متوسط)، و 5 جم MOS /كجم عليقه (مستوي مرتفع)، علي التوالي. استمرت التجربة لمدة ستة اسابيع. أظهرت النتائج أن إضافة المستوي المتوسط (3 جم MOS /كجم عليقه) أعطي أفضل أداء إنتاجي تمثل في تحسن معنوي في كل من الزيادة في وزن الجسم ونسبة التصافي وكذلك الأجزاء المأكولة من الطائر مقارنة بالمستويات الأخرى ، والمجموعة الضابطة. كما لوحظ أيضاً أن إضافة المستوي المتوسط من MOS أدى إلي زيادة البروتين الخام والرطوبة ، وانخفاض نسبة الدهن والرماد في لحوم السمان الياباني النامي. أوضحت النتائج أيضاً عدم وجود أي فروق معنوية في العد الكلي للبكتريا الهوائية وبكتريا الايشيريشيا كولاي في الأجزاء المختلفة لأمعاء السمان بين أي من المعاملات. كما أدى إضافة مستويات مختلفة من MOS إلى عليقة السمان إلي حدوث فروق معنوية في عدد عصيات الحليب في كلا من المعى الاثنا عشري والصائم حيث احدث المستوى المتوسط أعلى زيادة في عدد عصيات الحليب. وخلصت النتائج الي أن المستوي المتوسط (3جم MOS /كجم عليقه) أدى إلى تحسن مواصفات الذبيحة، جودة اللحم والبيئة الميكروبية لأمعاء السمان الياباني النامي عن طريق تثبيط البكتريا الممرضة وزيادة البكتريا النافعة.